In vivo glucose activation of the yeast plasma membrane ATPase

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The addition of glucose to yeast cells activates proton efflux mediated by the plasma membrane ATPase. Accordingly, the ATPase activity of purified plasma membranes is increased up to 10-fold. The activated ATPase has a more alkaline pH optimum, better affinity for ATP and greater sensitivity to vanadate than the non-activated enzyme. All these changes are reversed by washing the cells free of glucose. This suggests two states of the ATPase which are interconverted by a covalent modification. As glucose does not affect the phosphorylation of plasma membrane polypeptides, other type of covalent modification may be involved.

ATPase *Plasma membrane* *Yeast* *Proton transport* *Regulation* *Interconvertible enzyme*

1. INTRODUCTION

Yeast cells actively extrude protons in the presence of glucose and the phenomenon is blocked by inhibitors of the plasma membrane ATPase [1]. The participation of this enzyme in proton transport has been demonstrated by reconstitution of the purified ATPase in liposomes which catalyze ATP-driven proton transport [2,3]. The requirement for glucose observed in vivo was originally explained in terms of glycolysis providing the ATP utilized by the pump [1]. However, in yeast with high endogenous respiration glucose activates proton efflux without raising ATP concentrations (unpublished). In addition, the activity of the plasma membrane ATPase in homogenates from non-fermenting yeast ($1-2\ \mu mol \cdot min^{-1} \cdot g\ cells^{-1}$) is one order of magnitude lower than the initial rate of proton transport in glucose-fermenting yeast [1]. The possibility that glucose induces an activated state of the plasma membrane ATPase has been confirmed in this work.

2. METHODS

2.1. *Yeast and incubation conditions*

Commercial baker's yeast (Danubio, Madrid) was washed with water before use. The strain ATCC 42407 of *Saccharomyces cerevisiae* was grown to the stationary phase in a medium with 2% glucose and 0.5% yeast extract (Difco). After centrifugation, it was washed with water. Yeast cells (30–150 mg fresh wt/ml) were incubated at 30°C with mild agitation in a medium with 0.1 M 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 6.5 with Tris. When indicated, 0.1 M glucose was added and samples of 1–5 ml taken at the indicated times, frozen in a dry-ice–ethanol bath and stored at −70°C up to 3 days.

2.2. *Homogenization and membrane preparation*

All the operations were performed at 0–4°C. After thawing the yeast suspensions received 2 vol. glass beads (0.5 mm) and concentrated solutions of Tris and EDTA to reach 25 and 5 mM, respectively. The mixtures were vigorously vortexed for 2 min in a mixer (Lab-Line Instruments, Melrose

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Park IL) and the homogenates diluted 5-fold with a medium containing 6% sorbitol, 0.1 M Tris (adjusted to pH 8 with HCl), 5 mM EDTA and 2 mM dithiothreitol. After centrifugation for 3 min at 3000 rev./min the supernatants were decanted and centrifuged during 30 min at 30000 rev./min (Beckman 50 rotor). The pellets (total membrane fraction) were resuspended in a medium containing 20% glycerol, 10 mM *N*-[Tris(hydroxymethyl)methyl]glycine (adjusted to pH 7.5 with NaOH), 0.1 mM EDTA and 0.1 mM dithiothreitol. Yields ranged from 7–9 mg protein/g (fresh wt) of yeast. For the purification of plasma membranes 4 ml of total membranes were applied to discontinuous sucrose gradients formed by 3 ml of 53.5% sucrose (w/w) and 6 ml of 43.5% sucrose (w/w). After centrifugation for 6 h at 40000 rev.(min (Beckman SW 40 Ti rotor) plasma membranes were collected at the interphase, diluted with water, centrifuged for 30 min at 40000 rev./min and resuspended in the glycerol buffer described above. Yields ranged from 0.4–0.8 mg protein/g cells.

2.3. *ATPase assay*

The ATPase activity of total membranes (20–60 μg) or purified plasma membranes (3–15 μg) was determined in a medium containing 50 mM 2-(*N*-morpholino)ethanesulfonic acid (pH adjusted with Tris as indicated), 5 mM sodium azide (to inhibit mitochondrial ATPase), 0.2 mM ammonium molybdate (to inhibit acid phosphatase), 10 mM $MgSO_4$, 50 mM KCl and 0.1 mg/ml of egg yolk lysolecithin (Sigma type I; to destroy permeability barriers). The reaction was started with concentrated ATP to obtain the indicated final concentrations and the P_i liberated after 10 min at 30°C was determined as in [4].

2.4. *Protein determination*

Protein concentrations were determined by a modification of the Coomassie blue G-250 binding method [5] with bovine serum albumin as standard.

2.5. *^{32}P-Incorporation into membrane proteins and electrophoresis*

Baker's yeast (160 mg/ml) was incubated with 0.12 mCi/ml of carrier-free $^{32}P_i$ (Radiochemical Centre, Amersham) and after 2.5 h washed with 50 mM potassium phosphate (pH 6.5) and resuspended in water. After incubation for 5 min in the absence or in the presence of glucose, the cells were homogenized and the plasma membranes prepared as described above. SDS–polyacrylamide gel electrophoresis, slicing and counting of the gels were as in [6].

3. RESULTS

When yeast cells were incubated with glucose a rapid activation of the plasma membrane ATPase measured in homogenates was observed (fig.1). The activation was rapidly reversed after glucose removal and it was therefore essential to homogenize the glucose-fermenting cells without washing (see section 2). The reversible activation by glucose of plasma membrane ATPase was also observed in other yeast species like *Schizosaccharomyces pombe* and *Candida albicans* (not shown).

The activation of the ATPase seems to require glucose metabolism because it was prevented by

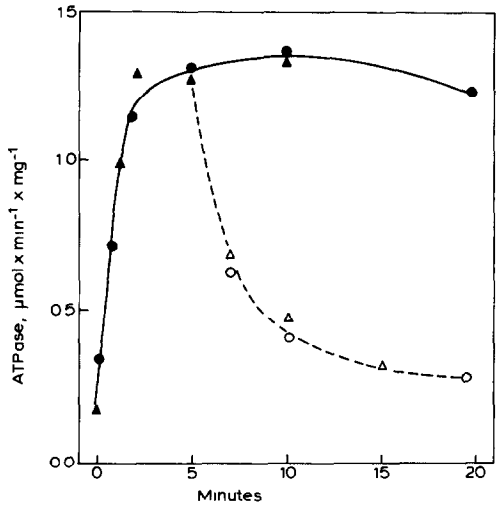

Fig.1. Reversible activation by glucose of the yeast plasma membrane ATPase. At time zero glucose was added to either commercial baker's yeast (triangles) or to the strain ATCC 42407 (circles) and samples were taken at the indicated times (closed symbols). After 5 min of incubation with glucose, part of the cells was washed by centrifugation in the cold and further incubated in the absence of glucose (open symbols). The ATPase activity of the total membrane fraction was determined at pH 5.7 with 2 mM ATP.

iodoacetate. In addition, the non-metabolizable glucose analogs D-xylose, 3-*O*-methylglucose and 2-deoxyglucose did not activate the ATPase. Fructose and mannose, which utilize the same glycolytic pathway as glucose [7], are also effective. On the other hand, galactose and ethanol were ineffective even in cells adapted to their utilization.

Cycloheximide (2 nmol/mg yeast) did not prevent the activation of the ATPase by glucose. Together with the rapidity of the phenomenon, this excluded synthesis of the ATPase protein as the mechanism of activation. As the activated state of the ATPase was preserved after purification of plasma membranes, it probably corresponds to a covalent modification of the enzyme.

The catalytic subunit of the ATPase (a major polypeptide of about 105000) and 3 major plasma membrane polypeptides of M_r 45000–55000 are phosphoproteins [8]. However, glucose fermentation did not change the phosphate content of any membrane polypeptide (not shown). Therefore, another type of covalent modification may be implicated in the phenomenon.

Other properties of the enzyme were modified by glucose. The glucose-activated ATPase has a pH optimum between 6 and 7 while the non-activated enzyme has a pH optimum of 5.6–5.7 and a shoulder between 6 and 7 (fig.2). The non-activated ATPase exhibited a K_m for ATP of 1.2 mM (strain ATCC 42407) or 2.0 mM (baker's yeast) while after glucose activation a value of 0.3 mM is observed in both cases (fig.3). The activated enzyme was more sensitive to vanadate than the non-activated one, with 50% inhibition at 3–5 μM and 17–18 μM, respectively (fig.4). Other properties like the sensitivity to diethylstilbestrol or the small activation produced by potassium

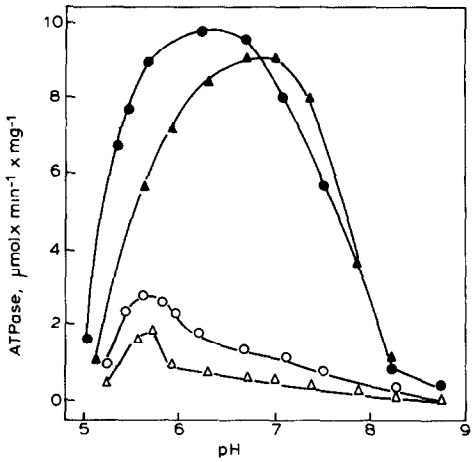

Fig.2. Effect of pH on the plasma membrane ATPase. Plasma membranes were purified from either commercial baker's yeast (triangles) or from the strain ATCC 42407 (circles) after 5 min of incubation in the presence (closed symbols) or in the absence (open symbols) of glucose. The ATPase activity was determined with 2 mM ATP.

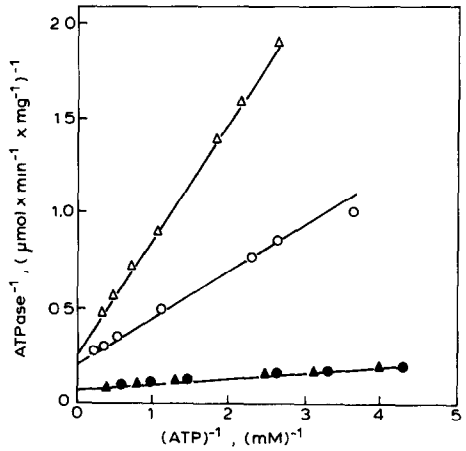

Fig.3. Double reciprocal plots of the effect of [ATP] on the plasma membrane ATPase. Symbols were as in fig.2. The ATPase activity was assayed at pH 5.7 and the [ATP] corresponded to the average concentration during 10 min reaction. ATP consumption was <20%.

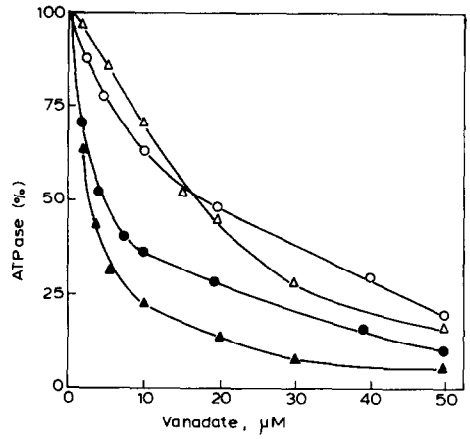

Fig.4. Inhibition of the plasma membrane ATPase by vanadate. Symbols as in fig.2. The ATPase activity was assayed at pH 5.7 with 2 mM ATP.

were not altered. All the changes in properties induced by glucose were reversed after washing the cells free of glucose.

To investigate the state of the ATPase during growth, the strain ATCC 42407 growing logarithmically on glucose, galactose or ethanol was quickly frozen in liquid nitrogen, homogenized and the plasma membrane isolated. The specific ATPase activities (μmol.min^{-1}.mg protein^{-1}; pH 5.7 and 2 mM ATP) were 7.5, 2.5 and 2.0, respectively. As the ATPase polypeptide represented 15–20% of the membrane protein in all cases, this suggests that the enzyme is in the activated state only during growth on glucose.

4. DISCUSSION

The activation of the plasma membrane ATPase described here may explain the requirement for preincubation with glucose to observe significant rates of active transport in yeast [9]. The electrochemical proton gradient generated by the plasma membrane ATPase is the driving force for active nutrient uptake and it has been observed [10] that preincubation with glucose hyperpolarizes yeast cells. Glucose preincubation also induces the synthesis of several transport systems [11] and this effect, which is blocked by cycloheximide, should also be taken into consideration.

The existence of two states of the enzyme may explain the disparity in kinetic properties reported for fungal plasma membrane ATPases [12]. The most conflicting results concern the specific activity, pH optimum, K_m for ATP, and the sensitivity to vanadate, which are precisely the properties modified by glucose.

The mechanism of the activation of the ATPase by glucose remains obscure. Glucose is known to increase the concentration of cAMP in yeast [13] and this activates a protein kinase which phosphorylates the enzymes trahalase [13] and fructose-bisphosphatase [14], altering their kinetic properties. Exogenous cAMP activates nutrient transport in some yeast strains [15,16], suggesting that it also mediates the effect of glucose on the ATPase. It is puzzling that I could not detect changes in the phosphorylation of the ATPase or other plasma membrane polypeptides after glucose addition to the cells.

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